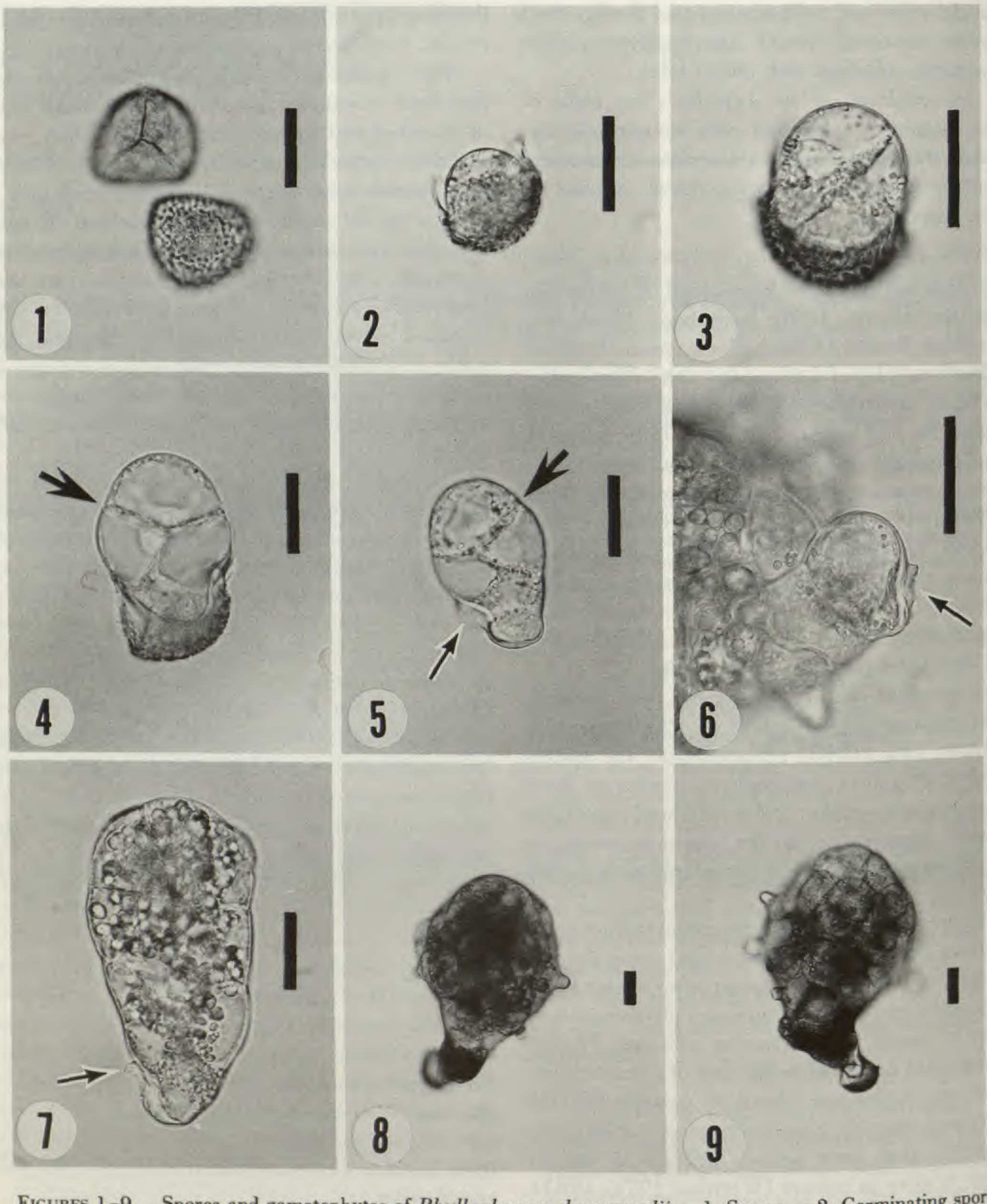
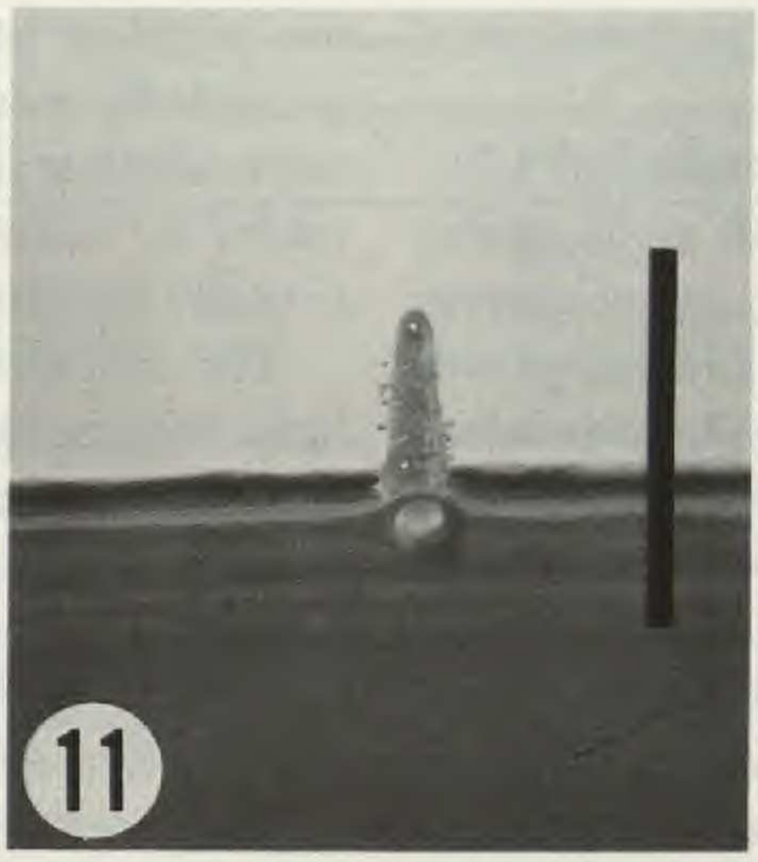
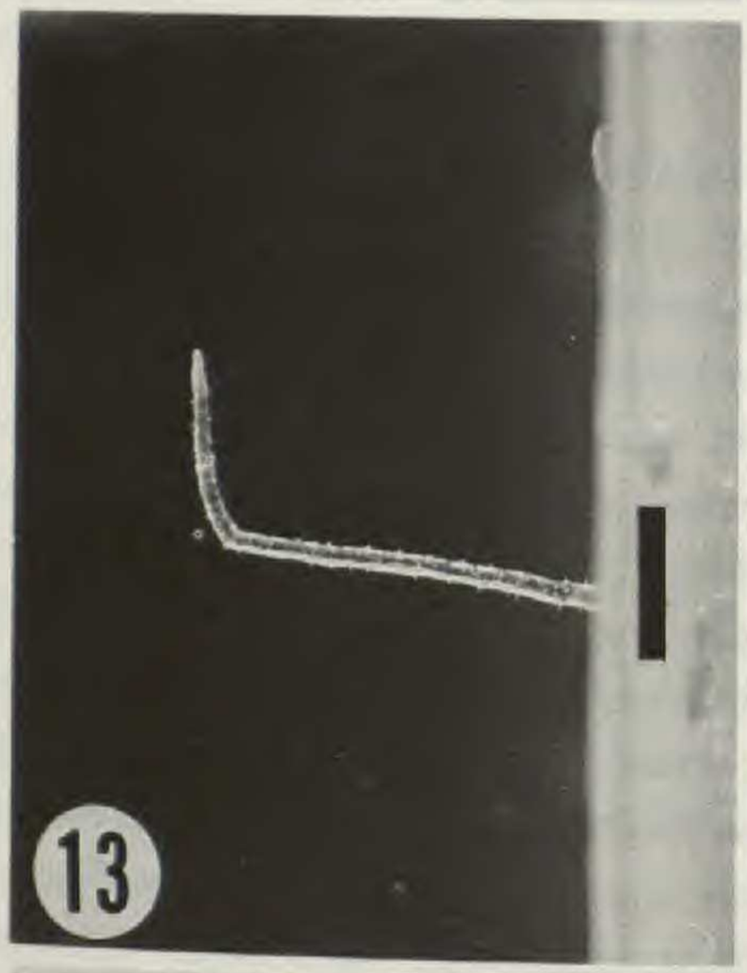


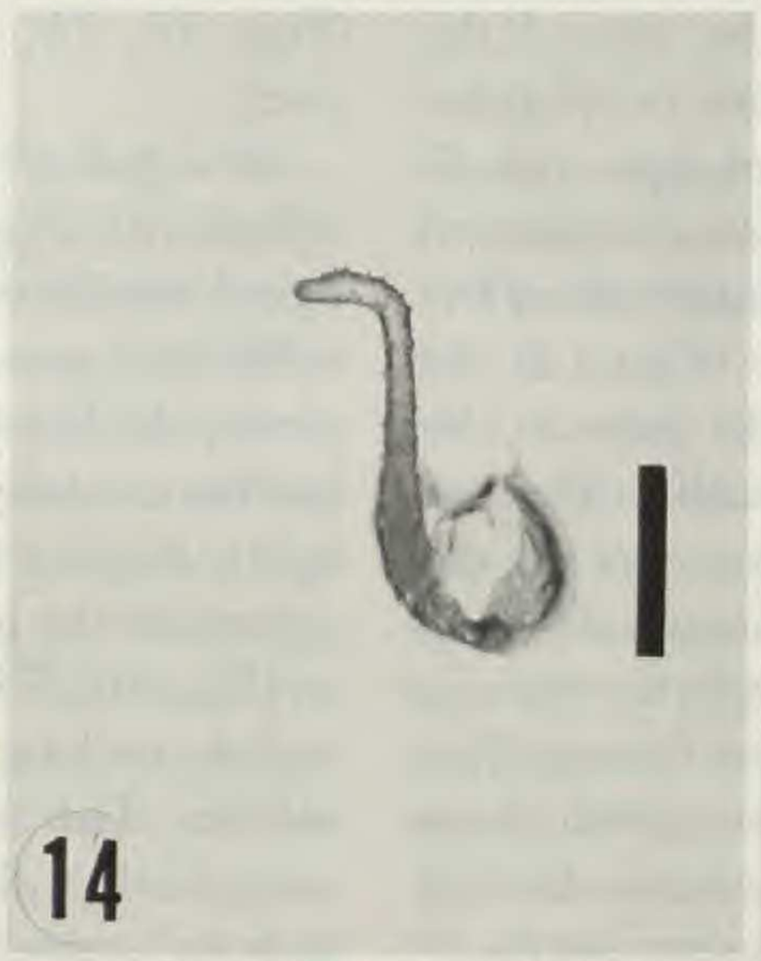
FIGURES 1–9. Spores and gametophytes of *Phylloglossum drummondii*.—1. Spores.—2. Germinating spore.—3. Two-celled gametophyte formed by oblique wall. Most of the distal cell and less of the proximal cell are in the spore coat.—4. Three-celled gametophyte with spore coat attached to distal cell. Arrow indicates the wall formed by second cell division of the gametophyte, which occurred in the proximal cell.—5. Three-celled gametophyte without spore coat. Note cytoplasm accumulated in base of distal cell. Small arrow indicates mucilage on surface of partially thickened wall of distal cell. Large arrow indicates cell wall formed by second cell division of gametophyte.—6. Distal end of multicellular gametophyte. Arrow indicates mucilage secretion by thickened portion of the wall of distal cell.—7. Small multicellular gametophyte. Arrow indicates mucilage and thickened wall of distal cell.—8, 9. Young globular gametophytes with young rhizoids and attached spore coats. All scale bars = 30 μm.

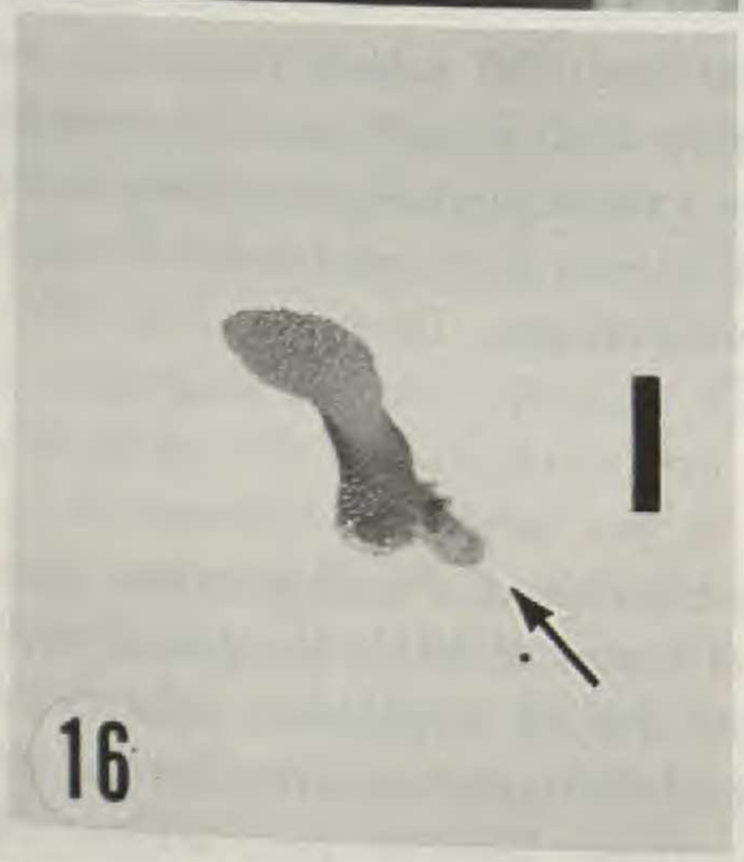
rows indicate new walls in Figs. 4, 5). The new wall produced by this division initially is almost perpendicular to the original wall formed by the first division. The distal cell does not undergo any more divisions (Figs. 6, 7).

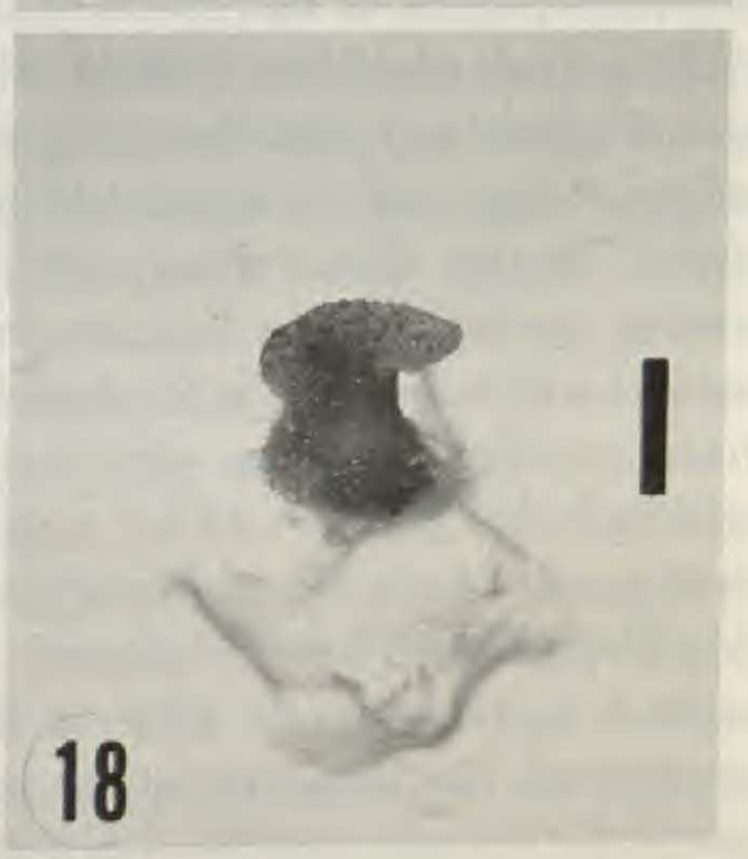
Wall differentiation of the distal cell becomes apparent at the 3-celled stage (Fig. 5). The distal portion of this wall thickens (Figs. 5–7). Usually in association with the wall thickening, either on it or adjacent to it, there is a secretion of muci-











FIGURES 10–18. Gametophytes of *Phylloglossum drummondii*.—10. Globular gametophytes.—11. Small vertically oriented cylindrical gametophyte with young rhizoids.—12. Gametophyte with globular base and apical cylindrical growth.—13. Cylindrical gametophyte with bend. New apical growth parallel to surface of nutrient medium (near scale bar) that was oriented vertically in the horizontal culture tube. New growth away from gravity.—14. Cylindrical gametophyte taken from reorientation experiment showing bending of gametophyte and new growth away from source of gravity.—15. Young photosynthetic gametophyte. Arrow indicates area at base of cylindrical portion without chlorophyll.—16. Young photosynthetic gametophyte. New tissue formed in light having a rough surface. Arrow indicates region at base of cylindrical portion without chlorophyll.—17, 18. Young photosynthetic gametophytes with horizontal orientation of the noncylindrical, new growth. All scale bars = 1 mm.

laginous material (Figs. 5–7; small arrows indicate mucilage). The mucilage stains with alcian blue at pH 2.6 (0.1% alcian blue in 3% acetic acid), which demonstrates that it contains an acid mucopolysaccharide.

The spore coat often remains attached to the distal cell until the gametophyte has attained a macroscopic size (Figs. 8, 9). It usually gets caught on the enlarging distal cell and deforms the surface of this cell. The deformity appears as a notch or

indentation in the wall where one edge of the spore coat is or was attached (Figs. 4–7).

Derivatives of the proximal cell undergo additional divisions to give rise to a small multicellular gametophyte (Fig. 7). The young gametophytes become globular with more divisions and cell growth (Figs. 8–10). At this stage rhizoids begin to develop.

If the gametophytes continue to grow in the dark, they become variously sized globular gametophytes. However, they eventually develop into cylindrical gametophytes (Figs. 11, 12), which grow up and away from the surface of the nutrient medium. The diameter of the cylindrical gametophytes appears to be related to the size of the globular gametophyte from which they develop because larger globular gametophytes give rise to thicker cylindrical gametophytes. The diameter of the globular gametophyte can be larger than the diameter of the cylindrical growth (Fig. 12). As long as the cylindrical gametophytes grow in the dark, they continue to grow vertically. The gametophytes tend to decrease in diameter as the apices get further from the nutrient medium. With some very long and narrow gametophytes the apical growth appears to be very slow or to stop. This appears related to the distances that carbohydrate has to move from the nutrient medium through the gametophytes to the apical growing points.

The growth of all the cylindrical gametophytes vertically and away from the surface of the nutrient medium suggested a negatively gravitropic response. To test this, cultures with gametophytes growing vertically were laid on their sides (horizontally) and maintained in the dark for five months. The apices of these gametophytes responded to the positional change by bending at right angles and growing away from the source of gravity (Figs. 13, 14). The gametophyte in Figure 13 was photographed in the culture tube at the end of the experiment. The direction of the new growth was parallel to the surface of the nutrient medium which had a vertical orientation in the horizontal culture tube (Fig. 13). The gametophyte in Figure 14, which shows the same reorientation to gravity, was removed from the culture tube to be photographed. Gametophytes in the reorientation test changed their direction of growth and became cylindrical gametophytes with right angle bends. These gametophytes demonstrate that the cylindrical gametophytes growing in the dark are negatively gravitropic.

Mature gametophytes of *Phylloglossum* are described as being green and photosynthetic (Thomas, 1901). However, the gametophytes in axenic culture maintain their white, cylindrical habit and do not produce gametangia in the dark. Cylindrical gametophytes were moved into the light to determine whether photosynthetic gametophytes can be raised in culture and whether they will become sexually mature.

The cylindrical gametophytes moved into the light become green (Figs. 15, 16). New growth from the apical region develops chlorophyll first, and this region is often darker green than older portions of the gametophyte. Chlorophyll development in the original cylindrical portions of these gametophytes is often slower. Sometimes the basal regions of these gametophytes never turn green (Figs. 15, 16; arrows indicate nongreen basal region).

New growth of the gametophytes in light is not cylindrical (Figs. 15–18) or vertical. A broader apical meristem replaces the conical apex of the cylindrical gametophyte. This broad apex forms a green, thickened gametophyte that grows more or less horizontally (i.e., perpendicular to the incident light). Besides the growth in length, the subapical regions of the green gametophyte widen and thicken (Fig. 16). The thickest part is the median dorsal region, making these areas hemispherical in cross section. The surface of the new growth is rough compared to the smooth surface of the white cylindrical gametophytes. Although these gametophytes are about one and a half years old, they are still immature and do not bear gametangia. Even though the gametophytes grow slowly in axenic culture, at this time they exhibit a shift toward their mature morphology.

Discussion

The best description of *Phylloglossum* gametophytes is by Thomas (1901). He described the gametophytes as having a primary tubercle, an upright cylindrical shaft, and an irregularly shaped photosynthetic crown bearing gametangia. The youngest gametophyte he described had a primary tubercle and an upright cylindrical shaft; the crown had not developed. He noted that the length of the shaft varied on mature gametophytes and that these shafts could be green near the crown; however, the ends near and including the primary tubercle were without chlorophyll. These gametophytes had a mycorrhizal fungus that was best developed in their lower portions. Finally, Thomas suggested that *Phylloglossum* gametophytes "may begin life as a saprophyte dependent on an endophytic fungus."

This description of gametophyte development in axenic culture is the first account of germination

TABLE 1. Character comparisons of *Phylloglossum* and subgenus *Lepidotis* of *Lycopodium*.

Character	*Phylloglossum*	*Lepidotis*
Basal mucilage duct in sporophyll	absent	present
Epidermal walls of sporangium	sinuate lignified	straight unlignified
Spore ornamentation	foveolate	rugulate
Spore germination	dark	light
Nutrition of young gametophyte	mycorrhizal	photosynthetic
Position of mature gametophyte	surficial	surficial
Nutrition of mature gametophyte	photosynthetic	photosynthetic
Photosynthetic lobes on gametophyte	absent	present
Multicellular uniseriate hairs on gametophyte	absent	absent
Embryo type	large foot	small foot

and early gametophyte growth for *Phylloglossum*. Prior to this report, the youngest gametophytes described were those with a primary tubercle and cylindrical shaft (Thomas, 1901). Bertrand (1885), Sampson (1916b), and Holloway (1935) failed to germinate the spores of *Phylloglossum*. An earlier and very brief report by Crié (1883) reported the germination of these spores but did not describe the early stages of gametophyte development. He did report mature, white, bulbous gametophytes that he considered to be similar to those of the Ophioglossaceae. Because the gametophytes he described do not fit the descriptions of gametophytes from nature (Thomas, 1901; Sampson, 1916b; Holloway, 1935) or from axenic culture, we choose to disregard the Crié report.

The 2-celled gametophyte of *Phylloglossum* consists of a proximal and distal cell. The distal cell undergoes no cell division. The most distal region of its cell wall thickens and secretes mucilage. At maturity the distal cell of these gametophytes is very similar to the proximal cell of young *Botrychium* gametophytes, which has a mucilage-coated, thick wall (Melan & Whittier, 1989). The proximal cell of the young *Phylloglossum* gametophyte with its thin wall remains meristematic. Through divisions of the proximal cell, the multicellular gametophyte is formed.

The globular gametophyte forms early in the development of *Phylloglossum* gametophytes in the dark. In axenic culture it usually does not become large before the initiation of the cylindrical gametophyte. However, in some cases the globular gametophytes are larger than the diameter of the cylindrical growths that arise from them. In these cases the gametophytes fit the description of a primary tubercle and cylindrical shaft described by Thomas (1901). Gametophytes moved to illuminated cultures appear to be initiating the gametophyte crown as described by Thomas (1901). Thus, morphological changes to the gametophytes in the light occur in both the soil and axenic culture.

The development of these gametophytes in culture is similar to that for gametophytes from nature. Growth of the gametophytes up through the soil to the light has to be simulated in culture by moving the gametophytes from the dark to the light. Also gametophytes in culture are dependent on sugar in the nutrient medium for their development and not on the mycorrhizal fungus necessary for gametophyte growth in nature.

Observations on the growth and development of *Phylloglossum* gametophytes in axenic culture can help to explain how they grow in nature. *Phylloglossum* spores germinate only in the dark, and the white cylindrical stage of these gametophytes is negatively gravitropic. It would appear that the spores in nature germinate only after being covered by soil or percolating into the soil. The early development of the white gametophyte requires a mycorrhizal fungus for its organic nutrition. Because the white cylindrical portion is negatively gravitropic, it grows up through the soil until it reaches the soil surface. Once the apex of the cylindrical gametophyte is exposed to the light, its developmental pattern changes and the photosynthetic, gametangia-bearing crown forms. Only at this stage is there any possibility for sexual reproduction.

The photosynthetic gametophytes and confusion about the tuber of *Phylloglossum* (Bower, 1886), led many to consider *Phylloglossum* as having affinities with *Lycopodium cernuum* or other members of subgenus *Lepidotis*. Also, Thomas (1901) described the embryo of *Phylloglossum* as being similar to that of *L. cernuum*. Although recognition that the tuber of *Phylloglossum* was not equivalent to the protocorm of *L. cernuum* (Sampson, 1916a; Osborn, 1919) has reduced the number of proposed similarities between *Phylloglossum* and subgenus

Lepidotis, *Phylloglossum* is generally considered to have affinities with subgenus *Lepidotis* (Holloway, 1935; Hackney, 1950; Bruce, 1976a).

In an effort to examine more closely any possible similarities between *Phylloglossum* and subgenus *Lepidotis*, a list of sporophytic and gametophytic characters are presented in Table 1. The sporophytic characters, which are few, do not include those used to classify *Phylloglossum* in the Lycopodiaceae or those altered by the reduced habit of *Phylloglossum*. Although a more exhaustive comparison awaits a complete description of the mature gametophyte of *Phylloglossum*, it appears that *Phylloglossum* and subgenus *Lepidotis* have few similarities (Table 1). The number of differences between *Phylloglossum* and subgenus *Lepidotis* suggests that a reexamination of past proposals of affinities between them is in order.

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